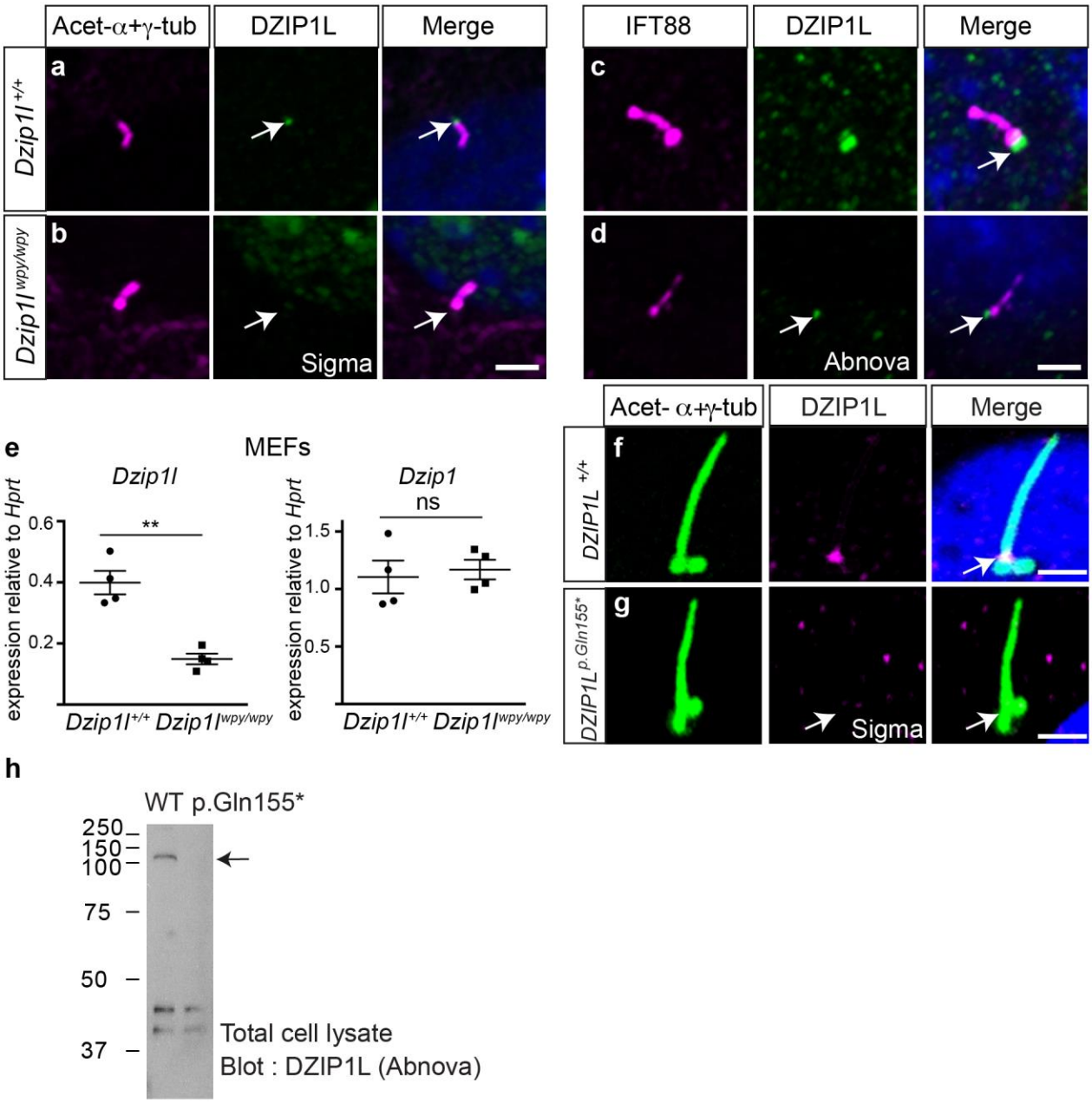
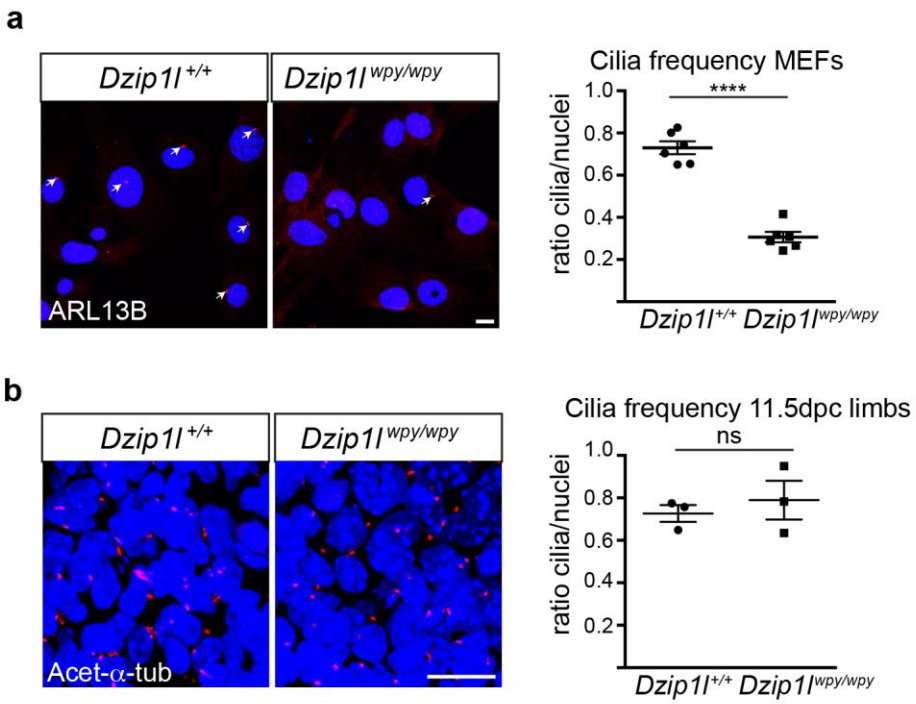


Supplementary Figure 7

Analysis of protein and transcript levels in *DZIP1L*-mutant cells.

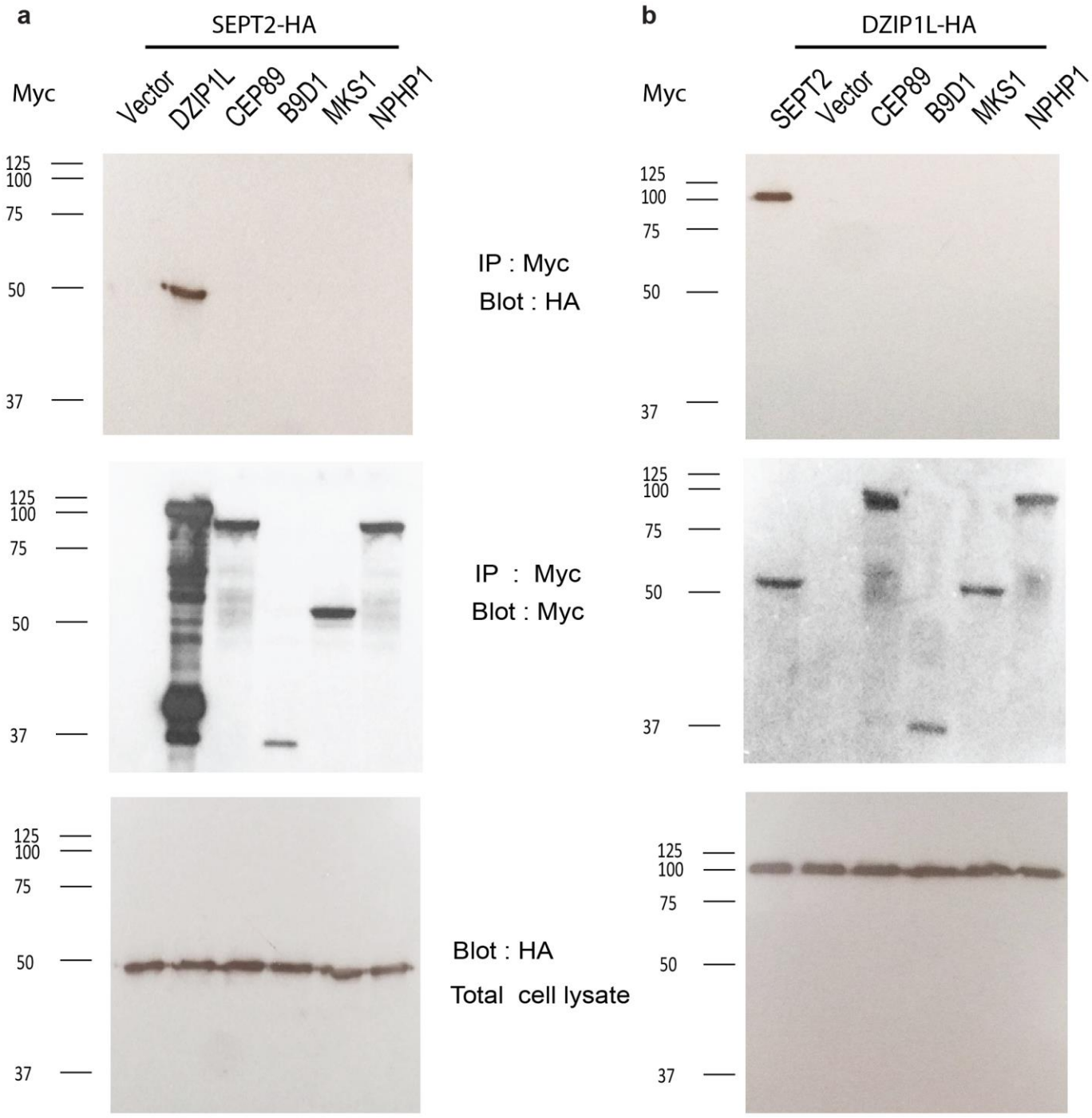
a,b) Staining with a C-terminal DZIP1L antibody (Sigma; green) reveals no signal in *Dzip1l*$^{wpy/wpy}$ MEFs (arrows in b), consistent with the truncated nature of the protein; (c,d,) Staining with an antibody raised to the entire DZIP1L protein (Abnova; green) reveals a residual level of truncated protein in *Dzip1l*$^{wpy/wpy}$ MEFs (arrows in d). The cilium is marked with acetylated α-tubulin and γ-tubulin in (a,b; magenta) and IFT88 in (c,d; magenta). (e) Based on an unpaired two-tailed Student's *t*-test, qRT-PCR revealed significantly reduced but not absent *Dzip1l* transcript levels in *Dzip1l*$^{wpy/wpy}$ MEFs relative to *Dzip1l*$^{+/+}$ wild-type MEFs, t(6)=5.905, **p=0.001 (n=4, consisting of 3 cell lines derived from independent embryos, analysed across two experiments (separate RNA preparations from one cell line analysed in both experiments); each data point is the average of 3 separate culture wells). *Dzip1* levels were not altered, t(6)=0.385, p=0.7135, ns, not significant, to compensate for reduced *Dzip1l*. Error bars, s.e.m. (f) DZIP1L (magenta) localizes at the transition zone in control human dermal fibroblasts, but is not detectable in *DZIP1L* mutant dermal fibroblasts from individual B155 (p.Gln155*) with the C-terminal Sigma antibody (arrows in g). Ciliary axonemes and basal bodies were labelled with anti-acetylated tubulin and γ-tubulin antibodies, respectively (green). (h) Immunoblotting with anti-DZIP1L antibody (Abnova) revealed a band corresponding to full length DZIP1L in control cell lysate (arrow) but not in lysate from DZIP1L mutant human fibroblasts. Scale bars in a-d = 2.5 m; g,h = 1μm.



Supplementary Figure 8

Dzip1l$^{wpy/wpy}$-mutant embryos show defects in ciliogenesis *ex vivo* but not *in vivo.*

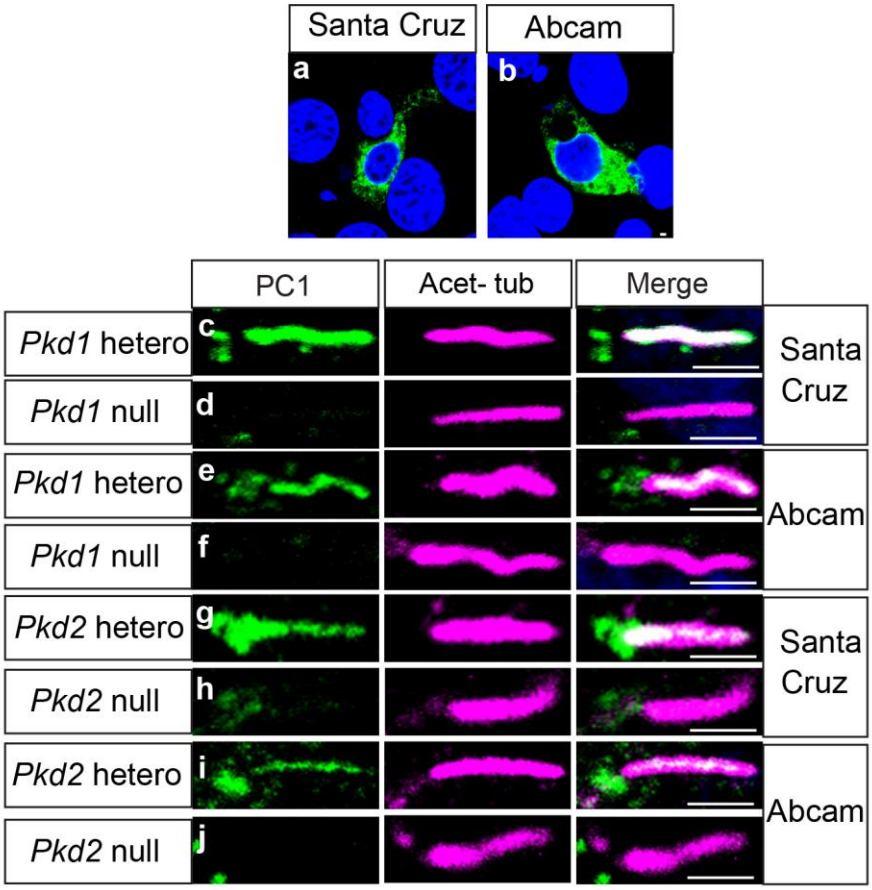
(a) Staining for cilia in serum-starved MEFs revealed a defect in the ability of isolated *Dzip1l*$^{wpy/wpy}$ mutant cells to ciliate relative to wild-type cells. Image and quantification based on ARL13B marking the cilium (red, arrows in a), but a significant reduction was also seen with acetylated-α-tubulin staining (n = 6 cell lines, each from an independent embryo; between 100-540 cells counted per cell line (1434 *Dzip1l*$^{+/+}$ and 1837 *Dzip1l*$^{wpy/wpy}$ cells in total), across two separate experiments). (b) Imaging and quantification of cilia in 11.5dpc forelimb buds following staining with acetylated-α-tubulin revealed no significant difference in cilia frequency (n = forelimb from 3 independent embryos, with 9 sections analysed per limb, >1900 cells counted per limb). Note the final cilia/nuclei ratio in limb sections will not necessarily represent the actual percentage of ciliated cells in the tissue, but is a relative value across the samples (see online methods for details). Statistical analysis based on an unpaired, two-tailed Student's *t*-test. (a) t(10)=10.87, ****p<0.0001; (b) t(4)=0.63, p=0.5624, ns, not significant. Error bars, s.e.m. Scale bar in a,b = 10μm. All data presented are from embryos on a C57BL/6:C3H mixed background.



Supplementary Figure 9

DZIP1L specifically associates with the ciliary-transition-zone protein SEPT2.

(a) Co-IP experiments from HEK293T cells transfected with expression constructs for epitope-tagged SEPT2 (C-terminal HA) and DZIP1L, CEP89, B9D1, MKS1 and NPHP1 (N-terminal Myc), reveal a specific interaction between SEPT2 and DZIP1L but none of the other proteins analysed. (b) Co-IP with DZIP1L (C-terminal HA) and SEPT2, CEP89, B9D1, MKS1 and NPHP1 (N-terminal Myc) confirmed the DZIP1L-SEPT2 interaction, and showed that none of the other proteins analysed interact with DZIP1L. Co-IP experiments were repeated three times.


Santa Cruz
Abcam
a
b
PC1
Acet- tub
Merge
Pkd1 hetero
Pkd1 null
Pkd1 hetero
Pkd1 null
Pkd2 hetero
Pkd2 null
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c
d
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f
g
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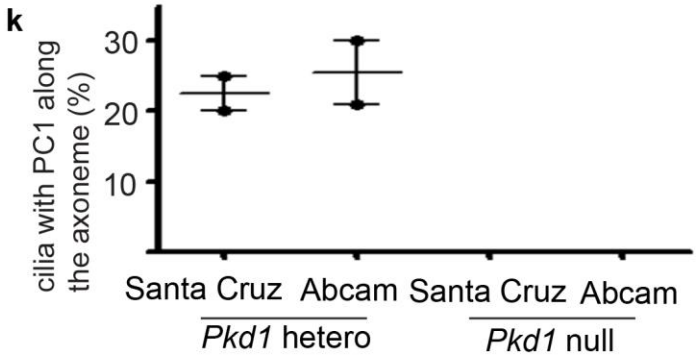
k
cilia with PC1 along the axoneme (%)
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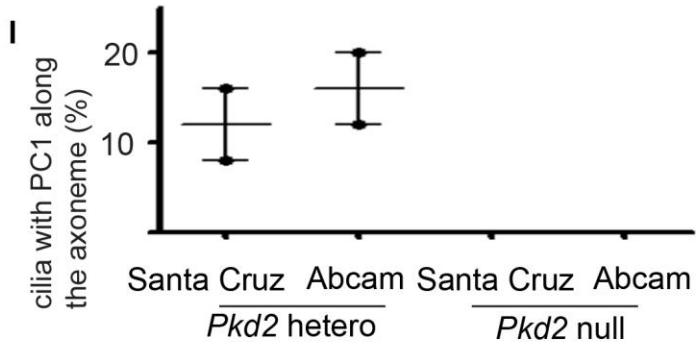
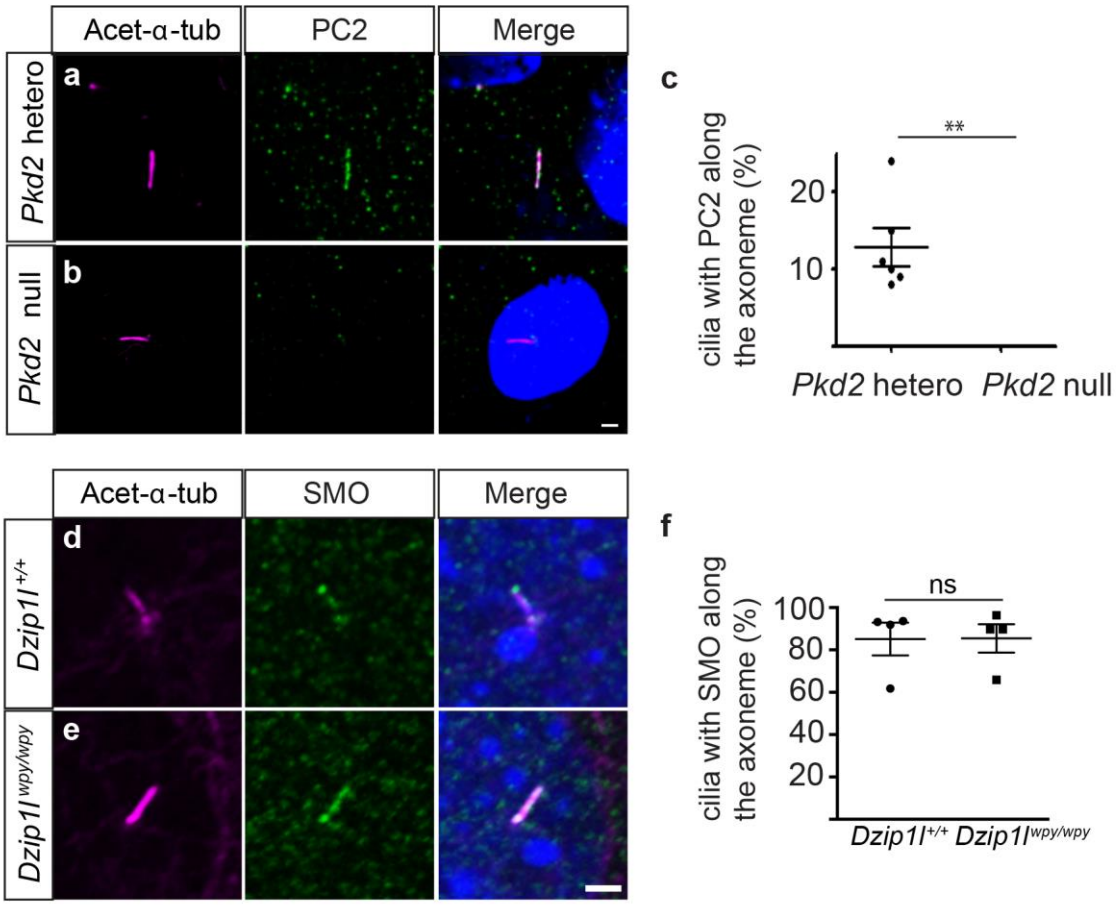
20
10
Santa Cruz
Abcam
Santa Cruz
Abcam
Pkd1 hetero
Pkd1 null


l
cilia with PC1 along the axoneme (%)
20
10
Santa Cruz
Abcam
Santa Cruz
Abcam
Pkd2 hetero
Pkd2 null

Supplementary Figure 10

Validation of the 7e12 antibody to PC1.

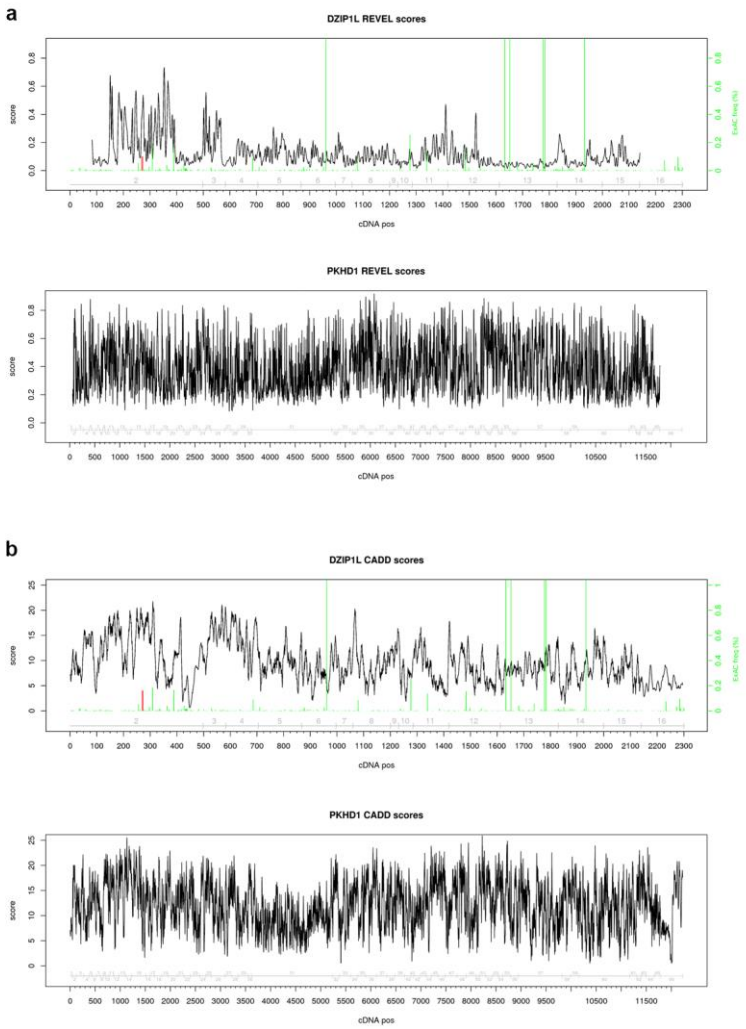
(a,b) PC1 antibody (mAb 7e12 raised to the N-terminal leucine rich repeat region of PC1 (24-180aa), aliquots obtained from both Abcam and Santa Cruz Biotechnology – see online methods for details) detected overexpressed PC1 protein (green) in IMCD3 cells. (c-j) PC1 staining (green) on the ciliary membrane in *Pkd1* or *Pkd2* heterozygote control cells, and lack of staining in null mutant cells, with the PC1 7e12 antibody preparation indicated. Acetylated-α-tubulin (magenta) marks the ciliary axoneme. (k,l) Quantification of the percentage of ciliated cells with PC1 along the axoneme in *Pkd1* or *Pkd2* heterozygous control cells. No homozygous mutant cells analysed displayed detectable PC1 staining along the axoneme. Note that PC2 is required for ciliary trafficking of PC1, so axonemal PC1 staining would not be expected in *Pkd2* null cells. Cilia were scored from two independent experiments. Approximately 100 ciliated cells were analysed in each experiment. The values are not compared statistically. Error bars, s.e.m. Scale bars = 1μm. See online methods for details of the *Pkd1* and *Pkd2* cell lines used for these validation experiments.



Supplementary Figure 11

Validation of antibody to PC2 and localization of SMO in *Dzip1l*$^{wpy/wpy}$ MEFs.

(a, b) PC2 staining (green) on the ciliary membrane in *Pkd2* heterozygote control but not null mutant cells with the PC2 antibody (MV12). Acetylated-α-tubulin (magenta) marks the ciliary axoneme. (c) Quantification of the percentage of ciliated cells with PC2 along the axoneme in *Pkd2* heterozygote and homozygote mutant cells. Statistical analysis based on an unpaired two-tailed Student's *t*-test, t(5)=2.57,**p=0.0033. Cilia were scored in three experiments, with cells from two independent coverslips analysed for each experiment (approximately 100 ciliated cells scored on each coverslip). (d,e) In response to treatment with the HH signalling agonist SAG, SMO (green) localizes along the axoneme (acetylated-α-tubulin, magenta) in both *Dzip1l*$^{+/+}$ and *Dzip1l*$^{wpy/wpy}$ MEFs. (f) quantification shows no difference in SMO localization between the two genotypes (n=4 MEF cell lines derived from individual embryos, analysed across two experiments; 28-91 cilia scored per cell line, total 299 *Dzip1l*$^{+/+}$ cilia and 137 *Dzip1l*$^{wpy/wpy}$ cilia scored). Statistical analysis based on an unpaired two-tailed Student's *t*-test, t(6)=0.0292, p=0.9776, ns, not significant. Error bars, s.e.m. Scale bars in a,b = 1μm; d,e = 2μm.



Supplementary Figure 12
Comparison of REVEL and CADD pathogenicity-prediction scores between *DZIP1L* and *PKHD1*.
A higher score indicates a more likely pathogenic effect of nucleotide exchanges at the corresponding position. For *DZIP1L*, red bars denote SNVs reported in this study, and green bars denote missense or loss-of-function variants found in ExAC. Height of green bars corresponds to the ExAC allele frequency in percent. (a) REVEL score. The x-axis refers to the coding DNA position of the longest transcript of the respective gene (*DZIP1L* = ENST00000327532; *PKHD1* = ENST00000371117). Exon numbers and bounds are drawn above the axis. The y-axis displays scores for all possible amino acid changes at the corresponding position, averaged with a scope of 4 bases to the left and right by loess smoothing (b) CADD score. The x-axis refers to the coding DNA position of the longest transcript of the respective gene (*DZIP1L* = ENST00000327532; *PKHD1* = ENST00000371117). Exon numbers and bounds are drawn above the axis. The y-axis displays scores for all possible amino acid changes at the corresponding position, averaged with a scope of 3 bases to the left and right by application of a Daniell kernel function.